

CHEMICAL CONSTITUENTS OF *HEDYOTIS HERBACEA*

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Kaempferol-3-O-rutinoside and ursolic acid were isolated from the leaves and twigs of Hedyotis herbacea. The structures of the two compounds were confirmed using spectroscopic techniques.

INTRODUCTION

Hedyotis herbacea is a small herb (0.1-0.2 m) usually bushy and commonly found in open places especially in sandy area. The plant which is also known as "tan ts'ao" is used as a poultice and sold in the Chinese herbalist shop (Burkill, 1936). Previous phytochemical studies on the genus *Hedyotis* showed the presence of flavonoid, iridoids, alkaloids and triterpenes (Ahmad Sazali Hamzah et al. 1994; Wu *et al.* 1991, Puroshothaman *et al.* 1981; Kikuchi et al. 1985). This paper reports the isolation and identification of two compounds from the extract of *Hedyotis herbacea*.

MATERIAL AND METHODS

Plant Samples

Hedyotis herbacea was collected from Tg. Tualang, Perak and was identified by Prof. Dr. Ruth Kiew of Department of Biology, University Pertanian Malaysia. A voucher specimen was deposited at the Herbarium of the Department of Biology, University Pertanian Malaysia.

Instrumentation

Melting points were determined on a Kofler hot-stage and were uncorrected. UV and IR spectra were obtained on Shimadzu Model 160 and Perkin Elmer Model 1600 spectrometers, respectively. ¹H- and ¹³CNMR were recorded on JOEL α 500 spectrometer. Mass spectra were obtained using Finnigan MAT Model SSQ 710 spectrometer and ionisation was effected by electron impact at 70 eV.

Column chromatography and analytical TLC were carried out using Merck 7729 and Merck DC-Plastikfollen 60 F₂₅₄.

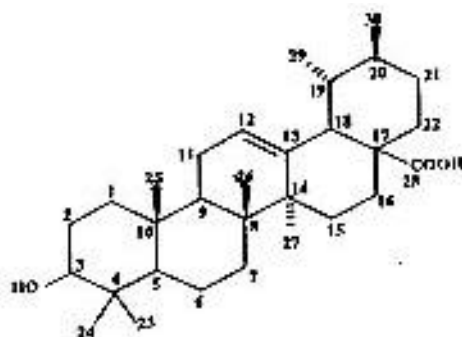
Extraction of Plant Materials

The leaves and twigs of *Hedyotis herbacea* (640 g) were soaked in methanol for forty-eight hours. The solvent was then removed under reduced pressure to give a dark green mass (30g). The crude methanol extract was then partitioned between chloroform and water. The aqueous layer was then extracted successively using ethyl acetate and butanol. The crude compounds were present in the chloroform, ethyl acetate and butanol fractions.

Isolation of ursolic acid (I)

The crude chloroform extract (3.5g) of *Hedyotis herbacea* was subjected to column chromatography using chloroform with increasing amount of methanol. Twenty-five fractions were collected, of which fractions 18-20 contained the major compound with a few minor constituents. The fractions were combined and then subjected to another column chromatography using ethyl acetate/chloroform (10:90) as the eluent. Ten 30 ml fractions were collected with fractions 4-7 containing the desired compound (45 mg). The compound obtained was a pale yellow powder with a melting point of 271-274 °c [lit. 266-267 °c (Takagi *et al.* 1979)]. The ^1H - and ^{13}C -NMR data of the compound were consistent with the reported data of ursolic acid (Takagi *et al.* 1979; Seo *et al.* 1975; Romeo *et al.* 1977; Kriwacki and Pitney, 1989).

UV λ_{max} (log ϵ) MeOH; 470 (0.14), 441 (1.18), 421 (0.17), 462 sh (0.13), 430 sh (0.16); IR ν cm^{-1} (KBr disk): 3750, 3432, 1692, 1540 1272, 1092, 996, ^1H NMR δ (500 MHz, CD_3OD): 5.21 (m, 1H, H-12), 3.14 (m, 1H, H-3), 2.20 (d, 1H, $J_{18,19} = 11.3$ Hz, H-18), 2.02-1.15 (m, 22H), 1.10 (s, 3H, C-23 Me), 0.96 (s, 3H, c-27 Me), 0.95 (s, 3H, c-26 Me), 0.93 (s, 3H, c-24 Me), 0.87 (d, 3H, c-29), 0.83 (d, 3H, c-30), 0.76 (s, 3H, c-25); ^{13}C -NMR δ (125 MHz, CD_3OD , DEPT-experiment): 181.6 (c-28), 139.6 (c-13), 126.8 (c-12), 79.6 (c-3), 56.7 (c-s), 54.3 (c-18), 47.6 (c-17), 47.6 (c-9), 42.8 (c-14), 40.7 (c-8), 40.4 (c-20), 40.4 (c-19), 39.9 (c-4), 39.8 (c-1), 38.1 (c-22), 38.1 (c-10), 34.3 (c-7), 31.7 (c-21), 29.2 (c-15), 28.7 (c-23), 27.8 (c-2), 25.3 (c-16), 24.3 (c-11), 24.0 (c-27), 21.5 (c-30), 19.4 (c-6), 17.8 (c-29), 17.6 (c-26), 16.3 (c-25), 16.0 (c-24); MS m/z (%): 456 (M^+), 248 (100), 219 (&.7), 207 (27), 203 (42.6), 189 (10.1), 133 (32.2), 119 (11.3), 69 (13.3).

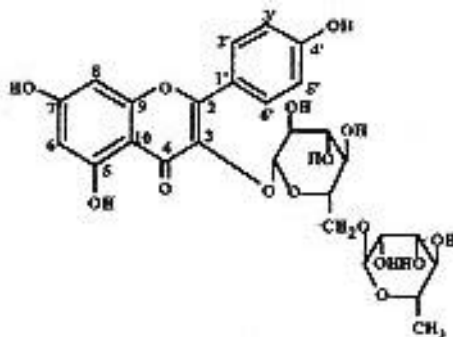


(I)

Isolation of Kaempferol-3-O-rutinoside (II)

Compound (II) was isolated from the crude butanol extract of *Hedyotis herbacea*. The crude butanol extract was subjected to gradient polarity column chromatography using ethyl acetate/methanol as the eluent. Twenty fractions were collected of which fractions 12-16 contained the major compound. The fractions were combined and subjected to another column chromatography using ethyl acetate/methanol (20:80) as the eluent. Twenty-five fractions were collected, of which fractions 14-20 showed a single spot on TLC and have same R_f values. These fractions were combined and recrystallised from methanol. The yellowish crystal has a melting point of 186-189°C [(lit. 210-215°C, Yu *et al.* 1991)].

UV λ_{\max} (log ϵ): 356 (0.66), 268 (0.89), 309 sh (0.50). IR $\nu_{\text{cm}^{-1}}$ (KBr disk): 3425, 2921, 1656, 1607, 1562 1510, 1451, 1063, 1044, 1014, 971, 912, 887, 835; $^1\text{H-NMR}\delta$ (500 MHz, CD_3OD): 8.06 (d, 2H, $J_{2',3'} = J_{6',5'} = 9.0$ Hz, H-2', 6'), 6.88 (d, 2H, $J_{3',2'} = J_{5',6'} = 8.7$ Hz, H-3', 5'), 6.40 (d, 1H, $J_{8,6} = 2.4$ Hz, H-8), 6.25 (d, 1H, $J_{6,8} = 2.3$ Hz, H-6), 5.12 (d, 1H, $J_{1'',2''} = 7.3$ Hz, H-1''), 4.57 (d, 1H, $J_{1'',2''} = 6.1$ Hz, H-1'''), 3.80 (dd, 1H, $J_{6a,6b} = 1$ Hz, $J_{6a,5} = 9.6$ Hz, H-6a), 3.61 (dd, 1H, $J_{2'',1''} = 1.7$ Hz, $J_{2'',3''} = 3.4$ Hz, H-2''), 3.53 (dd, 1H, $J_{6b,6a} = 3.6$ Hz, $J_{6b,5} = 9.5$ Hz, H-6b), 3.20-3.40 (m, 7H, H-2'', 3'', 4'', 5'', 3''', 4''', 5'''), 1.12 (d, 3H, $J_{6'',y'} = 6.1$ Hz, Rham- CH_3); $^{13}\text{C-NMR}$ δ (125 MHz, CD_3OD , DEPT- experiment): 179.4 (c-4), 166.3 (c-7), 163.1 (c-s), 161.6 (c-4'), 159.7 (c-9), 158.7 (c-2), 135.7 (c-3), 132.6 (c-2', 6'), 123.0 (c-1'), 116.4 (c-3, 5), 105.8 (c-10), 104.7 (c-1'''), 102.6 (c-1''), 100.3 (c-6), 95.2 (c-8), 78.1 (c-3'''), 77.3 (c-s'''), 75.9 (c-2'''), 74.1 (c-4''), 72.5 (c-3''), 72.3 (c-2''), 71.6 (c-4'''), 70.0 (c-5''), 68.8 (c-6'''), 18.1 (c-6''); Ms m/z (%): 286 (3), 279 (5), 167 (12), 149 (27), 125 (19), 111 (31), 97 (45), 85 (50), 71 (75), 57 (100).



(II)

RESULTS AND DISCUSSION

Extraction of the leaves and twigs of *Hedyotis herbacea* followed by extensive chromatographic techniques resulted in the isolation of ursolic acid and kaempferol-3-O-rutinoside. Ursolic acid, α -amyrin type triterpene was isolated from the crude chloroform extract of *Hedyotis herbacea*. The compound has a melting point of 271- 274 $^{\circ}\text{C}$ [(lit. 266- 267(c), Takagi et al 1979)]. The UV spectrum showed strong absorptions at 472, 444 and 421 nm and the IR spectrum exhibited strong absorptions at 3400 cm^{-1} (OH) and 1692 cm^{-1} (C=O). The mass spectrum showed a strong molecular ion peak at m/z 456 which corresponds to the molecular formula $\text{C}_{30}\text{H}_{48}\text{O}_3$. A base peak at m/z 248 is typical for α or β -type triterpenes. In order to distinguish between the two, carbon-13 values of the compound (Doddrell *et al.* 1974; Seo *et al.* 1975) was examined. The fundamental difference between the two triterpenes is at C-29 and C-30 of the ring E. In the α type triterpene (e.g. ursolic acid), both the methyl groups at ring E are secondary whereas in the β type (e.g. oleanolic acid) the two methyl groups are tertiary. By comparing the carbon-13 values of the compound against that of an α and β type triterpenes, the compound was found to show a closer resemblance to the α type.

The interpretation is further supported by the $^1\text{H-NMR}$ spectra of the compound. A doublet at 2.20 ppm with a J value of 11.3 Hz indicated that protons at C-18 and C-19 are trans to one another. This doublet only appears if the compound is of the α -type triterpene because the two groups attached to C-19 are hydrogen and methyl. The coupling between a single proton at C-18 and proton at C-19 could result in a doublet. On the other hand, if the compound is of the β -type triterpene (whereby only two protons are attached to C-19) the coupling between proton at C-18 and C-19 would give a quartet. A multiplet at 5.2 ppm which integrated for one proton is assigned to the olefinic proton of C-12 which is coupled to protons at C-11.

In addition to the above, peaks due to the aliphatic protons appear at higher field. Based on the spectroscopic data the compound is identified as ursolic acid.

The second compound, kaempferol-3-O-rutinoside (II) was isolated from the butanol extract of *Hedyotis herbacea*. The crude butanol extract was first subjected to dry column using ethyl acetate/methanol as the solvent. Fractions 3-5 were combined and then subjected to column chromatography using ethyl acetate/methanol (20:80) as the fluent. Twenty five fractions were collected of which fractions 15-20 gave the major compound. These fractions were combined and recrystallised from methanol. The yellowish crystal has a melting point of 186-189° C. The UV spectrum showed strong absorptions at 356 nm, 269 nm and 309 nm (sh) which is typical of a kaempferol skeleton (Markham, 1982). The position of the sugar unit was established by UV spectra in EtOH solution using NaOH (2M). Upon the addition of NaOH, the spectrum showed a bathochromic shift of 50 nm which indicates the presence of 4'-OH. The formation of a new band at 327 further indicates the presence of free 7-OH in the A ring of the kaempferol skeleton (Markham, 1982). The IR spectrum showed strong absorptions at 1656 cm^{-1} and 1607 cm^{-1} which correspond to the C=O and aromatic stretchings, respectively. A broad band at 2921-3425 cm^{-1} suggested the presence of hydrogen bonded OH group in the compound.

The $^1\text{H-NMR}$ spectra of the compound showed a typical kaempferol type with the presence of signals due to the sugar. Two doublets at 8.06 ppm and 6.88 ppm, both of which integrated for two protons, were due to protons of the B-ring. These protons signals appeared as two pairs of ortho coupled doubles with J values of 9.0 Hz and 87 Hz, respectively. Signals at 6.25 ppm (d, 1H, J = 2.3 Hz) and 6.40 ppm (d, 1H, J = 2.4 Hz) were due to the protons attached to C-6 and C-8, respectively. These protons, which are on the A-ring of kaempferol skeleton, have a low J values because they are meta to each other.

In addition to the above, strong signals also appeared at 5.12 ppm, 4.57 ppm, 3.10-4.0 ppm and a doublet at 1.12 ppm. Since these signals were complicated, a 2-D (COSY) technique was employed in order to assign the sugar protons. The signal at 5.12 ppm was coupled to protons at 3.30 ppm and from this observation, we deduced that the signal at 5.12 ppm was due to the isomeric carbon of a sugar which is coupled to protons attached to C-2. The signal, which was a doublet with J value of 6.8 Hz indicated that the linkage to the kaempferol skeleton is of β -type. A doublet at 4.57 ppm with J value of 2 Hz also suggested the existence of another isomeric proton in the compound with an α -linkage. This isomeric proton is again coupled to a sugar protons at 3.52 ppm. The high field signal at 1.12 ppm which integrated for three protons is

coupled to the signal at 3.32 ppm. This suggested that the methyl group is part of a sugar unit, a rhamnose. With the signals at the sugar region integrated for ten protons, hence, we could deduce the existence of two sugar units in the compound.

The ^{13}C NMR data further supported the presence of a kaempferol skeleton and the two sugar moieties. The low field signal at 179.4 ppm was due to the carbonyl group of C-4. The aromatic quaternary carbons of kaempferol skeleton appeared at 166.3 ppm (c-7), 163.1 (c-5), 161.6 (c-4'), 159.7 (c-9), 158.7 (c-2), 135.7 (c-3), 123.0 (c-1'), 105.8 (c-10), 100.3 (c-6) and 95.2 ppm (c-8). A high field signal at 18.1 ppm was due to the methyl group of the rhamnose unit. Two anomeric carbons of glucose and rhamnose appeared at 104.7 ppm and 102.6 ppm, respectively.

A methylene signal (CH_2) at 68.8 ppm was due to the C-6 of the glucose unit. Other signals due to the sugar residue occurred at 70-78 ppm. From the spectroscopic data and also from the comparison with previously published data, the compound is identified as kaempferol-3-O-rutinoside.

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